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SHORT COMMUNICATION

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Could natural selection change the geographic range limits of light brown apple moth (Lepidoptera, Tortricidae) in North America?

Amy C. Morey[1], Robert C. Venette[2], William D. Hutchison[1]

1 *Department of Entomology, University of Minnesota, St. Paul, MN, USA* **2** *Northern Research Station, USDA Forest Service, St. Paul, MN 55108, USA*

Corresponding author: *Robert C. Venette* (rvenette@fs.fed.us)

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We artificially selected for increased freeze tolerance in the invasive light brown apple moth. Our results suggest that, by not accounting for adaptation to cold, current models of potential geographic distributions could underestimate the areas at risk of exposure to this species.

Forecasting future distributions of invasive insects is important for many management and regulatory decisions. However, numerous challenges exist in creating accurate, biologically relevant models and maps that are meaningful over time (Venette et al. 2010). In particular, no models currently account for the potential of an invasive species to adapt to a new environment. In fact, demographic models in invasion biology commonly treat species as "homogenous immutable entities" (Lee 2002). For invasions by alien species in North America, adaptation to cold temperature may be especially important at northern latitudes or high elevations; cold often prevents species from surviving year round (Huey 2010, Venette 2013). For example, cold is likely to constrain the future distribution of the light brown apple moth, *Epiphyas postvittana* (Walker), a recent insect invader to North America. No model for *E. postvittana* currently accounts for the possibility of evolution of increased cold tolerance. The objective of this study was to determine if it was possible to artificially select for increased

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cold tolerance in this species, and if so, to begin exploring the subsequent geographical repercussions. *Epiphyas postvittana* is considered to be predominately freeze intolerant during its purported overwintering stage, the late instar larva (Burgi and Mills 2010), but preliminary data suggests that a small proportion of the population may also be freeze tolerant (Venette unpublished data). This phenomenon could be considered at least "partial freeze tolerance" (Sinclair 1999), and enabled freezing to act as a strong selection pressure for enhanced cold tolerance in our study.

Epiphyas postvittana eggs were obtained from USDA-APHIS (permit P526P-11-03713). All subsequent rearing and experimentation was conducted in a Biosafety Level 2 Containment Facility in St. Paul, MN. Eggs were held at 23°C, 60% RH, and resulting neonates were reared on artificial bean diet until late instars. We cooled 4-6th instars (verified through head capsule measurement; Danthanarayana 1975) individually inside gelatin capsules at ~1°C/min to their supercooling point inside a -80°C freezer (modified from Carrillo et al. 2004). Once freezing occurred, larvae were immediately returned to 23°C and given fresh diet. Mortality was measured as failure to eclose. Surviving moths were randomly mated in 0.47L (16 fluid oz) containers with 1-3 individuals of each sex. Randomly selected offspring were subsequently reared and supercooled as previously described. This procedure was repeated for nine generations. A minimum of 102 larvae were supercooled in each generation. A control population was maintained simultaneously and identically, save exposure to freezing.

Survival following freezing after nine generations was compared between the selected and control populations using non-parametric cumulative incidence functions (CIF) in SAS 9.3 (SAS Institute, Cary, N.C.) to address competing risk (Satagopan et al. 2004). The competing risk was any individual that froze and survived. These estimates were then used to calculate the temperature required to kill 50% (LT_{50}) of each population.

We used NAPPFAST (Magarey et al. 2009) to map where temperatures might fall below the LT_{50} of the selected or control populations. For each 10 × 10km grid cell, NAPPFAST calculated the proportion of the last 10 years in which the lowest temperature of the year was colder than the LT_{50} for each population. We ran the model with 3-D interpolated climate data.

After only nine generations of selection, the probability of survival following freezing was significantly (α=0.1) greater for the selected population than the unselected control (Fig. 1; P= 0.078, df=1, χ^2 =3.11). The LT_{50} for the unselected and selected populations were estimated to be –16.5°C and –19.0°C, respectively.

Figure 2 illustrates the geographic significance of a putative increase in cold tolerance for *E. postvittana*. Dark blue areas indicate the most dramatic effect of cold, where the LT_{50} was reached in 9-10 of the 10 years modeled. Cold was sufficient to exclude *E. postvittana* in many northern areas (e.g., Minnesota, the Dakotas, Wyoming, and much of Canada) using either the unselected (Fig. 2a) or selected (Fig. 2b) model. However, for other mid-western, eastern, and southern states, there was an overall reduction in the number of years where the LT_{50} was reached when using the selected population; the light blue to dark green colors shifted north. For example, the unselected model projected that nearly all of Michigan would reach the LT_{50} during 90–100% (dark

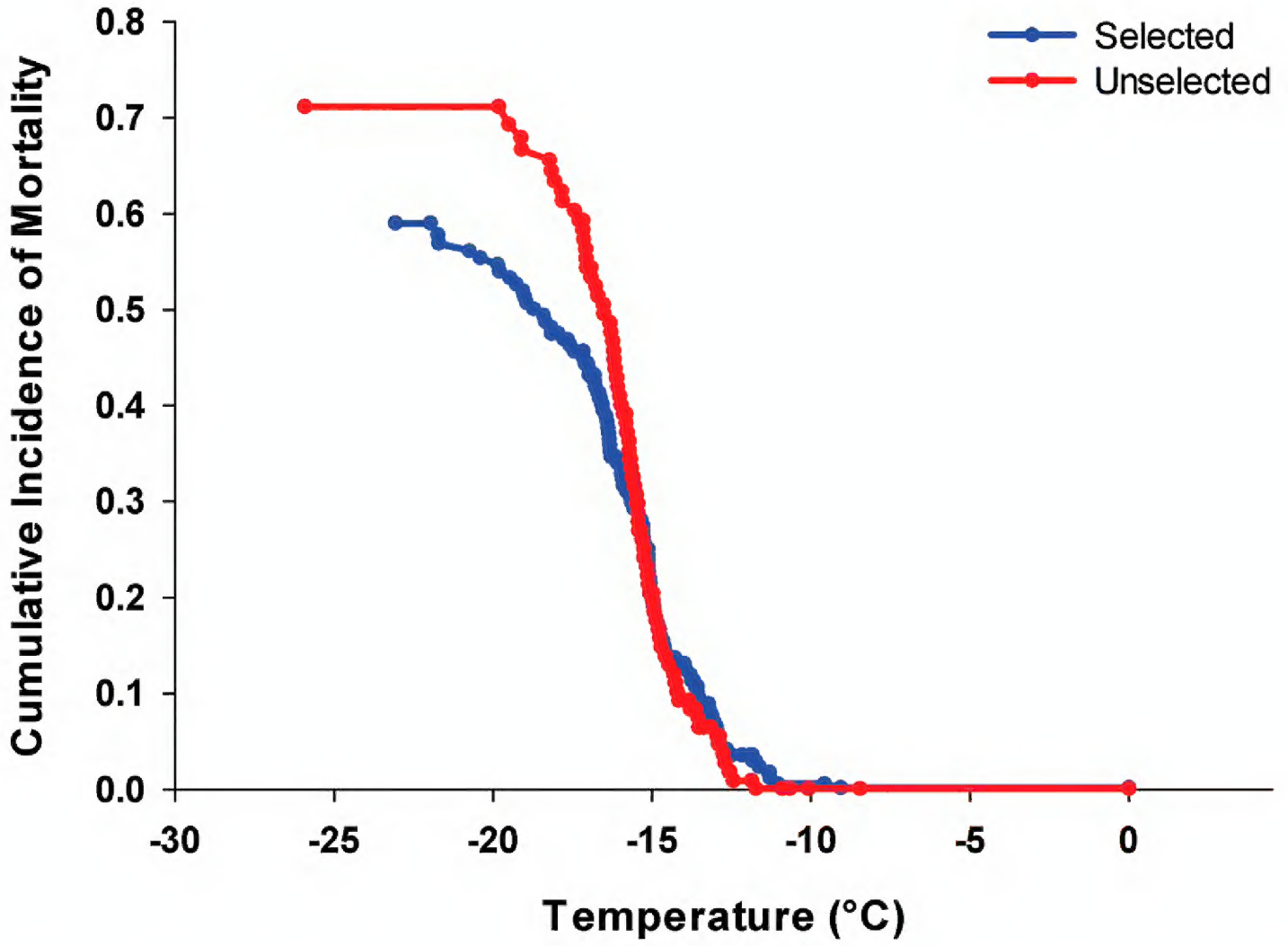

Figure 1. Cumulative incidence of mortality of *E. postvittana* late instars with (n=179) and without (n=109) nine generations of artificial selection for increased cold tolerance.

blue) of the years modeled. In contrast, the selected model projected that most of the state would only reach the threshold between 50 to 80% of the modeled years (light blue and white). Similarly, western states (e.g., Nevada and Idaho) showed an eastward shift in the number of years that temperatures did not reach the LT_{50}.

Current risk maps that exist for *E. postvittana* (e.g., Fowler et al. 2009, Gutierrez et al. 2010, Lozier and Mills 2011), acknowledge the importance of cold in shaping this species' potential U.S. range. However, the parameter(s) used to describe cold tolerance is/are assumed to be static. If natural selection follows a pattern similar to what our research suggests, within a relatively short period, current models may underestimate the risk of *E. postvittana* exposure in some areas in the future.

Uncertainty is inherent in pest risk models and contending with it is an ongoing area of research (Venette et al. 2010). Our study attempted to address the uncertainty related to the potential of a species to adapt to a cold environment and highlighted the geographic consequences if adaptation to cold is not considered. However, other sources of uncertainty still remain and are important future directions of this work. For example, the time of year may influence the effectiveness of selection on *E. postvittana*, assuming multi-voltinism and a randomly mating population. Selection is only likely to increase cold tolerance when there is a strong pressure (winter). But if there is any

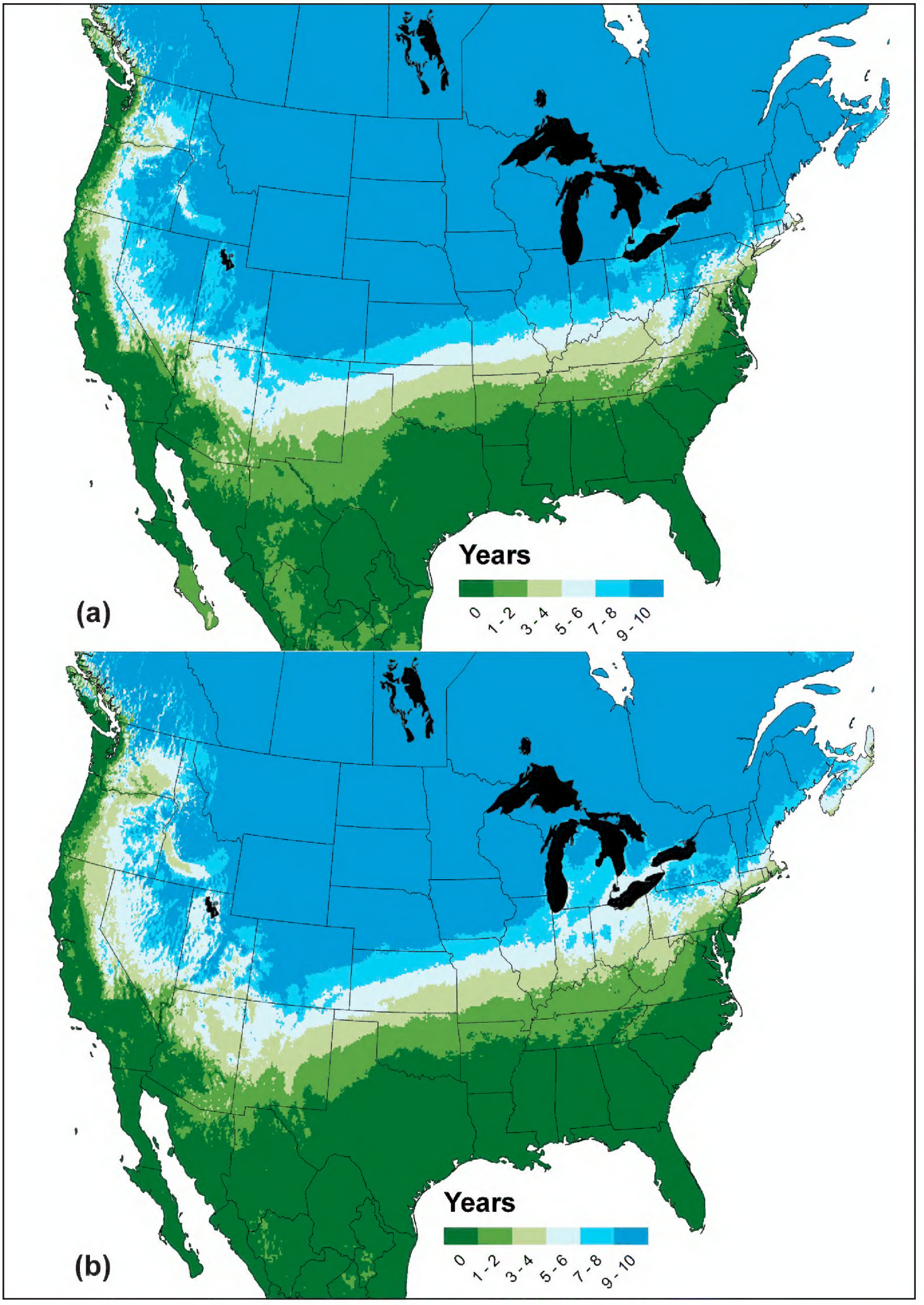

Figure 2. The frequency of years (2003–2012) in which temperatures fell below the threshold required to cause 50% mortality (LT_{50}) of *E. postvittana* in North America: **a** without selection, and **b** after nine generations of selection for increased freeze tolerance. Dark blue indicates where the LT_{50} was reached in 9–10 of the 10 years, dark green indicates where the LT_{50} was never reached during the 10-yr period.

trade-off between increased cold tolerance and fitness (see Watson and Hoffman 1996, Huey 2010), cold adapted individuals may be selected against during months when selection pressure is reduced (summer). Similarly, particularly for a highly polyphagous insect like *E. postvittana*, host plant variability could also affect cold tolerance measures (e.g., Liu et al. 2009). Understanding the relationship between cold tolerance and these additional factors will undoubtedly continue to further enhance the accuracy and ultimate utility of pest risk mapping tools.

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